

***Response to Restriction Requirement and Amendment******Page 2 of 5******Applicant(s): Emery et al.******Serial No.: 10/038,504******Confirmation No.: 5492******Filed: January 3, 2002******For: IMMUNIZING COMPOSITIONS AND METHODS OF USE***

Prior to taking up the above-identified application for examination, please amend the application as follows:

**In the Claims**

A 1 53. (Amended) The method of claim 47 wherein the at least two porins have molecular weights of between about 30 kDa and about 43 kDa.

A 2 61. (Amended) The method of claim 55 wherein the at least two porins have molecular weights of between about 30 kDa and about 43 kDa.

A 3 63. (Amended) A method for isolating outer membrane polypeptides, the method comprising:  
providing a gram negative microbe;  
disrupting the gram negative microbe in a buffer;  
solubilizing the disrupted gram negative microbe; and  
isolating molecules of the gram negative microbe, wherein the isolated molecules comprise outer membrane polypeptides comprising at least two SRPs and at least two porins, and LPS at a concentration of no greater than about 10.0 EU/ml.

64. (Amended) A method for isolating outer membrane polypeptides, the method comprising:  
providing a gram negative microbe;  
disrupting the gram negative microbe in a buffer, wherein the gram negative microbe is present in the buffer at a concentration of between about 720 grams of microbe per 1,000 milliliters of buffer and about 1,080 grams of microbe per 1,000 milliliters of buffer;  
solubilizing the disrupted gram negative microbe for greater than about 24 hours in a solution comprising sarcosine, wherein a ratio of the sarcosine to gram weight of disrupted

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gram negative microbe is between about 0.8 gram sarcosine per about 4.5 grams of disrupted gram negative microbe and about 1.2 grams sarcosine per about 4.5 grams of disrupted gram negative microbe; and

isolating molecules of the gram negative microbe, wherein the isolated molecules comprise outer membrane polypeptides comprising at least two SRPs and at least two porins.

*A 4*  
**66. (Amended) A method for isolating outer membrane polypeptides, the method comprising:**

providing a gram negative microbe;

disrupting the gram negative microbe;

solubilizing the disrupted gram negative microbe in a solution comprising sarcosine, wherein ratio of the sarcosine to gram weight of disrupted gram negative microbe is between about 0.8 gram sarcosine per about 4.5 grams of disrupted gram negative microbe and about 1.2 grams sarcosine per about 4.5 grams of disrupted gram negative microbe; and

isolating molecules of the gram negative microbe, wherein the isolated molecules comprise outer membrane polypeptides comprising at least two SRPs and at least two porins.

*A 5*  
**68. (Amended) A method for isolating outer membrane polypeptides, the method comprising:**

providing a gram negative microbe;

disrupting the gram negative microbe;

solubilizing the disrupted gram negative microbe for greater than about 24 hours;  
and

isolating molecules of the gram negative microbe, wherein the isolated molecules comprise outer membrane polypeptides comprising at least two SRPs and at least two porins.

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70. (Amended) A method for isolating outer membrane polypeptides, the method comprising:

providing a gram negative microbe;

disrupting the disrupted gram negative microbe in a buffer, wherein the gram negative microbe is present in the buffer at a concentration of between about 720 grams of microbe per 1,000 milliliters of buffer to about 1,080 grams of microbe per 1,000 milliliters of buffer;

solubilizing the gram negative microbe; and

isolating molecules of the gram negative microbe, wherein the isolated molecules comprise outer membrane polypeptides comprising at least two SRPs and at least two porins.

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